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DIFFERENCES IN THE FIELD ACTIVITY OF TWO FEMALE COLOR PHASES OF *COLIAS* BUTTERFLIES AT VARIOUS TIMES OF THE DAY

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INTRODUCTION

FEMALE *Colias eurytheme* butterflies generally exist in one or the other of two color phases—the "normal" orange and the dimorphic white form. The males are usually all of the same color. The white female phase is genetically differentiated from the orange by a dominant allele. The recessive allele for orange and the alternative dominant allele for white female color are carried in the autosomes of both males and females, but the white is manifested in the female only. All males are orange, irrespective of the alleles of this gene, which they carry.

Populations of *Colias* butterflies differ in the frequency of the dominant allele for white wing color. The frequency of this allele is higher in northern populations than it is in southern ones (Hovanitz, 1944*a*, 1944*b*) and also at higher elevations than at lower elevations (Hovanitz, 1945). The direct effects of the environment do not influence the manifestation of the character (Hovanitz, 1943*a*), and, therefore, it has seemed a logical deduction that the white-female allele provides a greater adaptive value to those butterflies carrying that allele in the areas where it is abundant. In wild populations the white females appear as adults in advance of the "normal" orange females in any given brood (Hovanitz, 1944*b*). This would seem to indicate a more rapid developmental rate for the white individuals in the examples which have been studied.

The geographical correlation existing between the color-phase frequencies and the specific ecological conditions already mentioned indicates a tendency of the two color-phase frequencies to be dependent upon the environment. If this is so, and if the environmental dependence is sufficiently great, then the color phases may indicate the nature of the dependence by differences in their response to the daily range of weather conditions to which each single population is subjected. It was for the purpose of testing this hypothesis that the following experiments were conducted.

The white female phase is composed of both the heterozygote and the dominant homozygote classes. There is no factual information available concerning a difference in adult diurnal behavior of these two classes or in the frequencies of the two dominant groups in a population which is in genetic equilibrium, as shown by the formula $p^2 + 2pq + q^2$ where p equals the dominant allelic frequency and q equals the recessive allelic frequency, nor is there any evidence that these same differences may be ascribed to the three equivalent genotypes present in the males, in which they are not visibly differentiated.

ACKNOWLEDGMENTS

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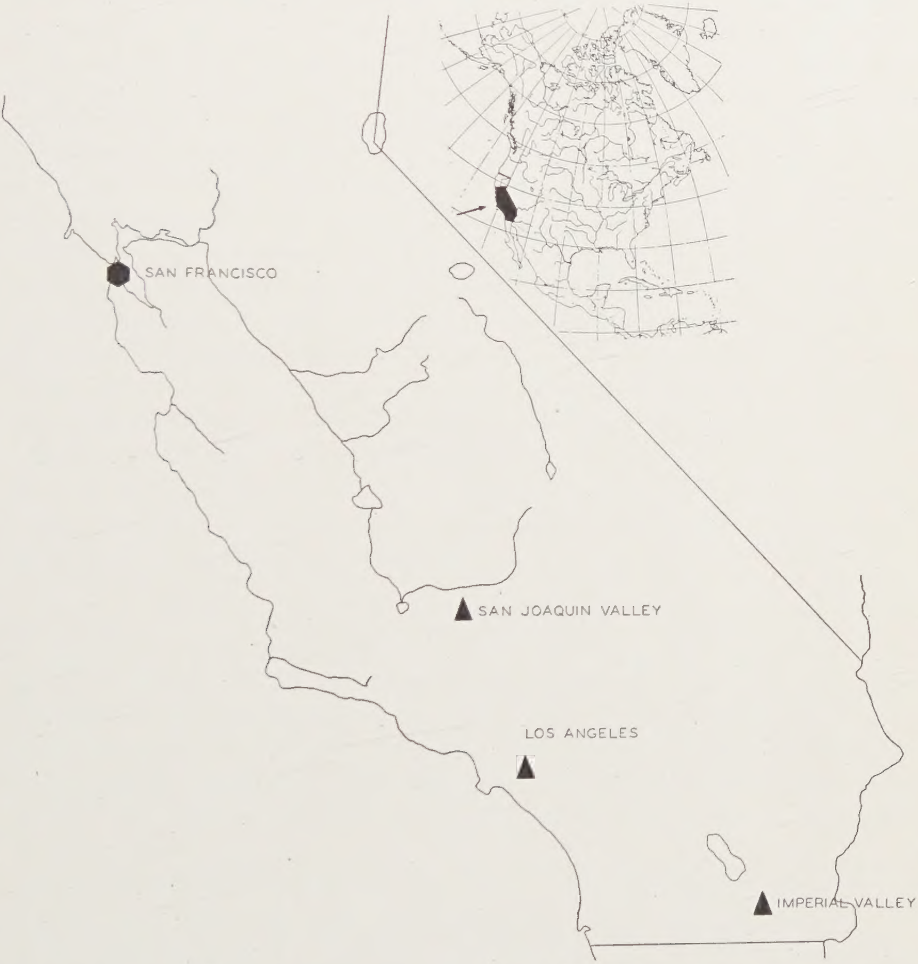
METHODS

The differential activity of the polymorphic female forms during the day was measured in the "species" or "race" *Colias eurytheme* (or *chrysotheme*). In this species the males are normally orange, and some of the females may be orange and others may be a very contrasting white.

The localities chosen for the study were in southern California. Here all populations are restricted to the host-plant alfalfa (*Medicago sativa* or lucerne) and are free from any intercrossing with related species of *Colias*. The numbers of individuals in a given restricted population are large, thus making the accumulation of significant data rather simple. In addition the variations in meteorological phenomena during the day are more dependable in the West than they are in Eastern areas, where conflicting air mass movements play a proportionally greater part in the daily weather changes.



MAP 1. The locations (triangles) of the three alfalfa fields in which the diurnal changes in color-phase frequencies were recorded.

The analyses of the field behavior of the two color phases in *Colias eurytheme* (the alfalfa butterfly) were made in three different places. The first analysis, in September, 1941, was made near Los Angeles, California (Rio Hondo). The second was in June, 1946, near Bakersfield, San Joaquin Valley, California. The third was also in June, 1946, but near Brawley, Imperial Valley, California. These three localities are shown in relation to each other on Map 1. Each population studied was in a single alfalfa field. In the second and third studies, the fields averaged about one-half by three-quarter miles in area; the field in the first study was about 200 by 300 feet in area.

The first analysis differed from the other two in that each butterfly was captured, marked, and released. The manner in which this was done is being described elsewhere (Hovanitz, MS). In the latter two analyses no butterflies were captured; instead, the white and orange females were counted as the observer walked rapidly up and down the field. The speed of the walk was determined partly by the abundance of butterflies. Whereever a cloud of butterflies rose from the field upon being disturbed, it was necessary for the observer to walk slower.

The counts in a field were made as nearly as possible at random. Only in those rare cases in which a single butterfly might have been counted a number of times in succession by flying directly ahead of the observer was any deviation made in the path of the observer. The direction of the path was changed to avoid such an individual if it persisted in the same direction, or the observer stopped momentarily while the butterfly flew away. These were only rare cases. In general the counts were made while the observer walked up and down the field starting from a corner of the rectangular area and at each change in direction shifting at right angles a distance equivalent to two irrigation "checks." Each irrigation check was about thirty feet wide. This procedure was continued until the termination of the time interval as given in the data.

In all three of these analyses no butterflies were removed permanently from the fields by capture. Over a period of time, therefore, the same individuals may have been counted several times. The frequency of recapture would be governed by the laws of chance. In the first analysis the butterflies were marked as to time and place. Therefore, the frequency of recounted individuals may be determined from these data. This phase of the study is to be presented in another paper (Hovanitz, MS).

DAILY VARIATION IN THE FREQUENCIES OF THE COLOR PHASES

The females observed in an alfalfa field show different frequencies of the white and the orange phases in the population at different times of the morning and afternoon. The change in frequencies is consistent from day to day, from hour to hour, and from population to population when similar factors of the weather are compared.

A high frequency of white females is evident early in the day, followed by a lower frequency in the middle of the day and by a rise again in the afternoon under some circumstances. This curve of variation seems to be controlled by physical factors of the environment and to be, therefore, directly correlated with the variations of the latter.

The first analysis was made in September, 1941, near Los Angeles, California (Rio Hondo). For three days the female individuals of *Colias* in this field (see Map 1) were caught, marked, and released, thus indicating the frequency of the forms in the field (Table I). Each of the three days, September 20, 21, and 22, was divided into three parts. In the first part of the day were included all those females caught before 10:30 or 10:45 A.M. In the second part were included those caught between 10:30 A.M. and 12:00 noon (or 12:45 P.M.), and in the third part were included those caught after 12:00 noon. The collections generally began about 8:30 A.M. and lasted until about 3:00 or 3:30 P.M.

It may be observed from the totals for the three days that there has been a change in the frequency of the two color phases which were flying during the day. The first part of the day has a frequency of 27.5 ± 3.5 per cent white females. This has dropped to 22.4 ± 3.9 per cent white in the second part and has risen again to 34.1 ± 4.1 per cent white in the third part of the day. The difference between the first two parts of the day (5.1 ± 5.14 per cent) has a probability value of about .50 of being due to chance alone. The difference between the second and third parts of the day (11.7 ± 5.7 per cent) has a probability of about .05 of being due to chance alone. Taken in this way, the data indicate a change which might have a fair probability of not being due to chance alone. The results of the three days, however, may be calculated separately, and the probability value may be determined for the morning rise in frequency, the noon drop, and the afternoon rise. The chi-square test has been used here, based upon daily averages in frequencies. Deviations from the expected average frequency are obtained from numerical values adjusted to the proportion of the total individuals taken during a part of the day, with seven degrees of freedom, $p = 0.90$–0.95 that the differences might be greater if due to chance alone. A similar chi-square test made for the combined three parts of the day with the expected values adjusted to the average frequency for the three days gives $p = 0.10$–0.20 that the differences might be due to chance alone (two degrees of freedom).

Because of the desirability of knowing definitely whether the low significance values were due to the small sample sizes only, and whether or not a real change was involved, analyses two and three in the San Joaquin and Imperial valleys, respectively, were made.

The second analysis was made near Bakersfield, San Joaquin Valley, California, at a place arbitrarily called Base (map in Hovanitz, 1944*b*). In 1942 two test samples at this place indicated a white female frequency of 52.68 ± 1.28 per cent and 53.73 ± 2.51 per cent. The frequencies in this field have been determined at different times of the day on June 3, 6, and 7, 1946, with the results summarized in Table II. It should be observed that these data were collected by nearly continuous counts during the day as shown by the time intervals included. They were grouped into time

TABLE I

THE FREQUENCIES OF WHITE TO ORANGE FEMALES IN A SINGLE SUBPOPULATION OF *Colias eurytheme* AT DIFFERENT TIMES OF THE DAY
Rio Hondo, Near Los Angeles, California. (Analysis 1)

Date	Part 1				Part 2			
	Before 10: 30 A.M.*				10: 30 A.M.–12: 00 Noon†			
	Orange	White	*N*	Per Cent White	Orange	White	*N*	Per Cent White
Sept. 20, 1941	35	20	55	36.4 ± 6.5	27	9	36	25.0 ± 7.2
Sept. 21, 1941	35	9	44	20.5 ± 6.1	35	11	46	23.9 ± 6.3
Sept. 22, 1941	46	15	61	24.6 ± 5.5	28	6	34	17.6 ± 6.5
Total	116	44	160	27.5 ± 3.5	90	26	116	22.4 ± 3.9
	Part 3				Total			
	After 12:00 noon							
	Orange	White	*N*	Per Cent White	Orange	White	*N*	Per Cent White
Sept. 20, 1941	60	33	93	35.5 ± 5.0	122	62	184	33.7 ± 3.5
Sept. 21, 1941	29	13	42	31.0 ± 7.1	99	33	132	25.0 ± 3.8
Sept. 22, 1941	......	......	......	....	74	21	95	22.1 ± 4.3
Total	89	46	135	34.1 ± 4.1	295	116	411	28.2 ± 2.2

* To 10 : 45 A.M. on Sept. 21 and Sept. 22.

† To 12 : 45 P.M. on Sept. 22 ; from 10 : 45 A.M. on Sept. 21 and Sept. 22.

TABLE II

THE FREQUENCIES OF ACTIVE WHITE FEMALES OF *Colias eurytheme* IN AN ALFALFA FIELD NEAR BAKERSFIELD, SAN JOAQUIN VALLEY, CALIFORNIA, AT INTERVALS DURING THE DAY
(Analysis 2)

Time	Approximate Class Center	*N*	White	Per Cent White	*S. E.* Per Cent
June 3, 1946					
8: 05 A.M.— 9: 10 A.M.	8: 35 A.M.	284	153	53.87	± 2.95
9: 25 A.M.—10: 00 A.M.	9: 45 A.M.	249	122	49.00	± 3.17
10: 30 A.M.—11: 25 A.M.	11: 00 A.M.	199	96	48.24	± 3.87
11: 40 A.M.—12: 55 P.M.	12: 15 P.M.	143	72	50.34	± 4.18
June 6, 1946					
8: 50 A.M.— 9: 55 A.M.	9: 25 A.M.	431	220	51.04	± 2.41
10: 00 A.M.—11: 10 A.M.	10: 35 A.M.	441	245	55.56	± 2.37
11: 30 A.M.—11: 55 A.M.	11: 45 A.M.	177	82	46.33	± 3.75
12: 00 Noon– 1: 05 P.M.	12: 30 P.M.	298	145	48.66	± 2.89
1: 30 P.M.— 2: 30 P.M.	2: 00 P.M.	189	83	43.92	± 3.61
3: 00 P.M.— 4: 35 P.M.	3: 45 P.M.	420	203	48.33	± 2.44
5: 05 P.M.— 6: 15 P.M.	5: 35 P.M.	348	183	52.59	± 2.68
June 7, 1946					
6: 55 A.M.— 7: 45 A.M.	7: 20 A.M.	203	121	59.61	± 3.34
7: 50 A.M.— 8: 30 A.M.	8: 10 A.M.	161	87	54.04	± 3.83
10: 00 A.M.—11: 10 A.M.	10: 35 A.M.	527	272	51.61	± 2.17
12: 30 P.M.— 1: 30 P.M.	1: 00 P.M.	263	110	41.83	± 3.04
1: 40 P.M.— 2: 25 P.M.	2: 00 P.M.	222	85	38.29	± 3.26
3: 55 P.M.— 4: 55 P.M.	4: 25 P.M.	325	172	52.92	± 2.77
5: 00 P.M.— 5: 55 P.M.	5: 30 P.M.	237	128	54.01	± 3.14

classes for the purpose of calculating the significance of any change which might be apparent between the different times. A mean frequency for each time class was calculated and for these classes the standard error was computed. The mean percentages of white females flying over the field have been plotted against time to make a chart which graphically shows the changes that have occurred in the population during the day (Fig. 1).

On June 3 the sampling was of limited duration, and only a minor change in the color phase frequencies occurred. On June 6 a general drop in white frequency extended through the morning until nearly 2:00 P.M. Then there was a rise of the white frequency again which did not quite reach the original level.

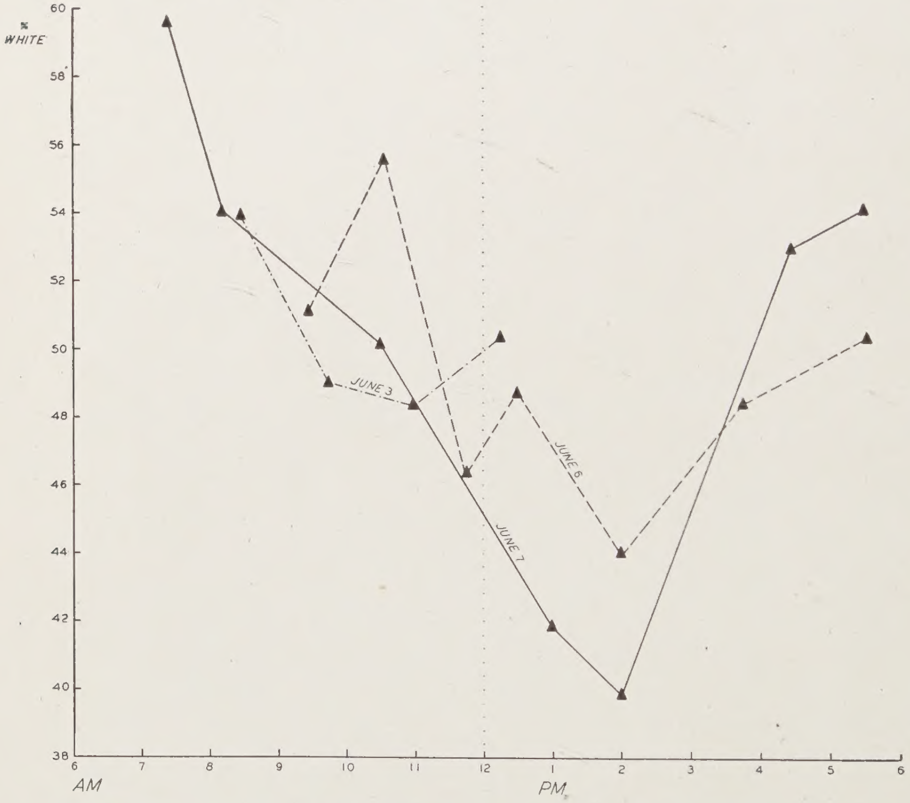


FIG. 1. The frequencies of the white-phase females in an alfalfa field near Bakersfield, San Joaquin Valley, California, at different times during the three days, June 3, 6, and 7, 1946.

On June 7 larger numbers were obtained, and the sampling was continued over the more extreme parts of the daily weather variations. On this day the changes in color phase frequencies were most marked, ranging from nearly 60 per cent white at 7:00 A.M. to a low of 40 per cent white at 2:00 P.M. and then to a high again of 54 per cent at 5:30 P.M. The differences

TABLE III

The Frequencies of Active White Females of *Colias eurytheme* in an Alfalfa Field Near Brawley, Imperial Valley, California, at Intervals During the Day
(Analysis 3)

Time	Approximate Class Center	*N*	White	Per Cent White	*S. E.* Per Cent
June 9, 1946					
12:40 P.M.— 1:40 P.M.	1:00 P.M.	744	138	18.55	±1.42
3:00 P.M.— 3:50 P.M.	3:25 P.M.	810	144	17.78	±1.34
5:15 P.M.— 6:10 P.M.	5:35 P.M.	754	134	17.78	±1.36
June 10, 1946					
5:55 A.M.— 6:50 A.M.	6:25 A.M.	1,113	264	23.72	±1.27
8:05 A.M.— 9:00 A.M.	8:30 A.M.	1,158	226	19.52	±1.16
10:05 A.M.—11:00 A.M.	10:30 A.M.	1,043	175	16.78	±1.15
12:05 P.M.— 1:15 P.M.	12:40 P.M.	967	167	17.27	±1.19
2:30 P.M.— 3:40 P.M.	3:05 P.M.	1,036	178	17.18	±1.14
5:20 P.M.— 6:25 P.M.	5:50 P.M.	1,310	267	20.38	±1.01
June 11, 1946					
5:35 A.M.— 6:55 A.M.	6:15 A.M.	1,388	359	25.86	±1.38
8:00 A.M.— 9:00 A.M.	8:30 A.M.	1,405	289	20.57	±1.07
9:55 A.M.—10:55 A.M.	10:25 A.M.	839	143	17.04	±1.30

TABLE IV

THE RELATIVE NUMBERS OF ACTIVE *Colias eurytheme* FEMALES COUNTED IN AN ALFALFA FIELD NEAR BAKERSFIELD, SAN JOAQUIN VALLEY, CALIFORNIA, AT INTERVALS DURING THE DAY

Time	Approximate Class Center	Minutes Included	*N*	Number Per Minute		
				Total	White	Orange
June 3, 1946						
8: 10 A.M.— 9: 10 A.M.	8: 40 A.M.	65	266	4.09	2.23	1.86
9: 25 A.M.— 9: 55 A.M.	9: 40 A.M.	35	227	6.49	3.11	3.37
10: 35 A.M.—11: 25 A.M.	11: 00 A.M.	55	180	3.27	1.56	1.71
11: 40 A.M.—12: 50 P.M.	12: 15 P.M.	75	140	1.87	0.95	0.92
June 6, 1946						
8: 55 A.M.— 9: 55 A.M.	9: 25 A.M.	65	415	6.38	3.29	3.09
10: 00 A.M.—11: 05 A.M.	10: 35 A.M.	70	418	5.97	3.39	2.59
11: 35 A.M.— 1: 00 P.M.	12: 15 P.M.	90	444	4.93	2.36	2.58
1: 35 P.M.— 2: 25 P.M.	2: 00 P.M.	55	166	3.02	1.35	1.67
3: 05 P.M.— 4: 30 P.M.	3: 45 P.M.	90	392	4.36	2.11	2.24
5: 10 P.M.— 6: 10 P.M.	5: 40 P.M.	66	316	4.86	2.62	2.25
June 7, 1946						
7: 00 A.M.— 7: 45 A.M.	7: 25 A.M.	50	187	3.74	2.22	1.52
7: 50 A.M.— 8: 25 A.M.	8: 05 A.M.	40	129	3.23	1.73	1.50
10: 05 A.M.—11: 10 A.M.	10: 35 A.M.	65	474	7.29	3.78	3.51
12: 35 P.M.— 1: 25 P.M.	1: 00 P.M.	50	222	4.44	1.70	2.74
1: 45 P.M.— 2: 20 P.M.	2: 05 P.M.	40	195	4.88	1.75	3.13
4: 00 P.M.— 4: 55 P.M.	4: 25 P.M.	60	312	5.20	2.82	2.38
5: 00 P.M.— 5: 50 P.M.	5: 25 P.M.	55	217	3.95	2.09	1.85
Average per minute	....	......	....	4.586	2.298	2.289

TABLE V

The Relative Numbers of *Colias eurytheme* Females Counted in an Alfalfa Field Near Brawley, Imperial Valley, California, at Intervals During the Day

Time	Approximate Class Center	Minutes Included	*N*	Number Per Minute			
				Total	White	Orange	White × 4.043
June 9, 1946							
12: 45 P.M.— 1: 35 P.M.	1: 05 P.M.	55	714	12.98	2.44	10.55	9.86
3: 05 P.M.— 3: 45 P.M.	3: 25 P.M.	45	712	15.82	2.93	12.89	11.85
5: 20 P.M.— 6: 05 P.M.	5: 45 P.M.	50	703	14.06	2.46	11.60	9.95
June 10, 1946							
6: 00 A.M.— 6: 45 A.M.	6: 25 A.M.	50	990	19.80	4.80	15.00	19.41
8: 10 A.M.— 8: 55 A.M.	8: 35 A.M.	45*	1,072	23.82	4.71	19.11	19.04
10: 10 A.M.—10: 55 A.M.	10: 35 A.M.	50	937	18.74	3.14	15.60	12.70
12: 10 P.M.— 1: 10 P.M.	12: 40 P.M.	65	983	13.58	2.35	11.23	9.50
2: 35 P.M.— 3: 35 P.M.	3: 05 P.M.	65	922	14.18	2.49	11.69	10.07
5: 25 P.M.— 6: 20 P.M.	5: 55 P.M.	60	1,157	19.28	3.95	15.33	15.97
June 11, 1946							
5: 40 A.M.— 6: 50 A.M.	6: 15 A.M.	75	1,256	16.75	4.48	12.27	18.11
8: 05 A.M.— 8: 55 A.M.	8: 30 A.M.	55	1,245	22.64	4.64	18.00	18.76
10: 00 A.M.—10: 50 A.M.	10: 25 A.M.	55	759	13.80	2.35	11.45	9.50
Average per minute				17.121	3.395	13.726	

* Five minutes lost by interruption.

between these extremes are very significant, and a change in color phase frequencies in the active population which was available for counting is indicated.

The third analysis was made near Brawley, Imperial Valley, California (Map 1). In this valley the average white female frequency during 1942 (Hovanitz, 1944*b*) was 16.31 ± 0.82 per cent. The variation at different times of the three days, June 9, 10, and 11, was studied in 1946 in a single alfalfa field (Table III). It must be observed again that the data as shown are mean values for the time intervals during which the samples were being taken. Here, as contrasted with the San Joaquin Valley population, the sampling was not continuous, but was interrupted with rest periods. Owing to the character of the weather, a continuous walk of more than twelve hours during the one day was not considered feasible. A continuous sampling was not necessary because a sample size of approximately one thousand females was relatively easy to obtain in an hour.

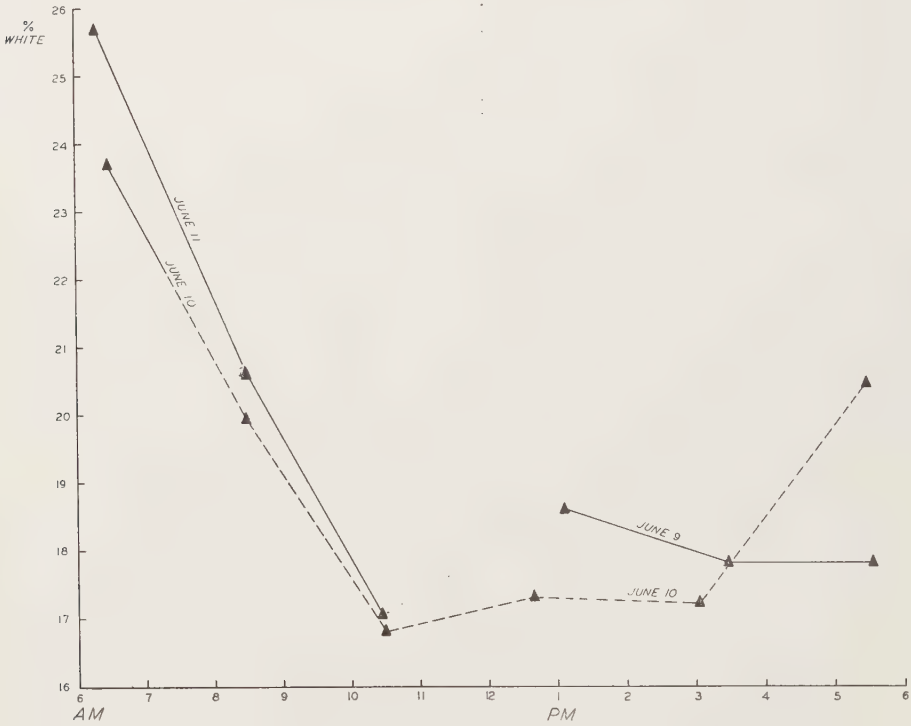


FIG. 2. The frequencies of the white-phase females in an alfalfa field near Brawley, Imperial Valley, California, at different times during the three days, June 9, 10, and 11, 1946.

The mean frequencies for the white females at the nearly two-hour intervals are plotted against time in Figure 2. It may be observed in Figure 2 that on the afternoon of June 9, the white female frequency did not change from the constant low of about 18 per cent white. Nevertheless, on the morning of June 10, the first sample indicated 24 per cent white; at 10:30 a.m. a minimum of between 17 and 18 per cent was white. This percentage was comparable to that of June 9. It stayed at this minimum until late in the afternoon, when it rose a little (to 21.5 per cent) just before sunset. The following morning it was again high (around 25.8 per cent) and dropped as before to a low at 10:30 A.M. Owing to the large numbers involved in these samples, the differences are very significant, and the probability of the random occurrence of such changes is negligible.

The following fluctuations occurred. In each population a high white frequency was present early in the morning and a low white frequency toward the middle of the day. The minimum of white was not reached at the same time in the two different localities. In the Imperial Valley the minimum was reached by 10:30 A.M., after which there was no further change. In the San Joaquin Valley the minimum was not reached until 2:00 P.M.; then a rise was immediately apparent. This fact may indicate that a minimum of selective effect of the environment on the activity of the two color phases was reached in the Imperial Valley which may not have been reached in the San Joaquin Valley. Further discussion of these fluctuations, however, requires the introduction of information on the total activities of the butterflies and also on the relative activities of the two color phases themselves.

VARIATION IN ACTIVITY OF THE FEMALES

The method of sampling which has been used gives a rating of the comparative activity of the females at different times of the day. At all times

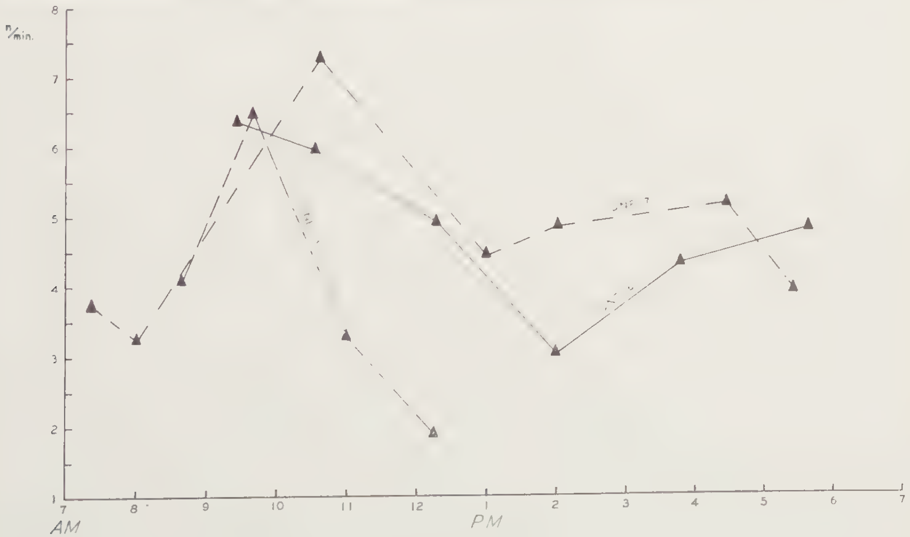


FIG. 3. The average number of female *Colias* counted per minute at various times of the day in the San Joaquin Valley alfalfa field.

the observer was attempting to count as many females as possible while in the field. He was limited in this in two ways. The first limitation was the distance between him and the butterfly when the number of active adults was low. In order to discriminate well between males and orange or white females it was desirable to be within ten yards of the individuals. When adults were rare, the numbers counted could be increased per unit of time by walking faster. This was done, but it was possible only up to obvious limits. When adults were very common, the difficulty in indicating the full increase lay in the identification of the polymorphic types in a swarm that would rise from the field as the observer walked by. The upper and lower limits to the relative abundance figures which have been obtained are, therefore, decreased and increased respectively by the nature of the technique and do not represent absolute values for determining variations in activity.

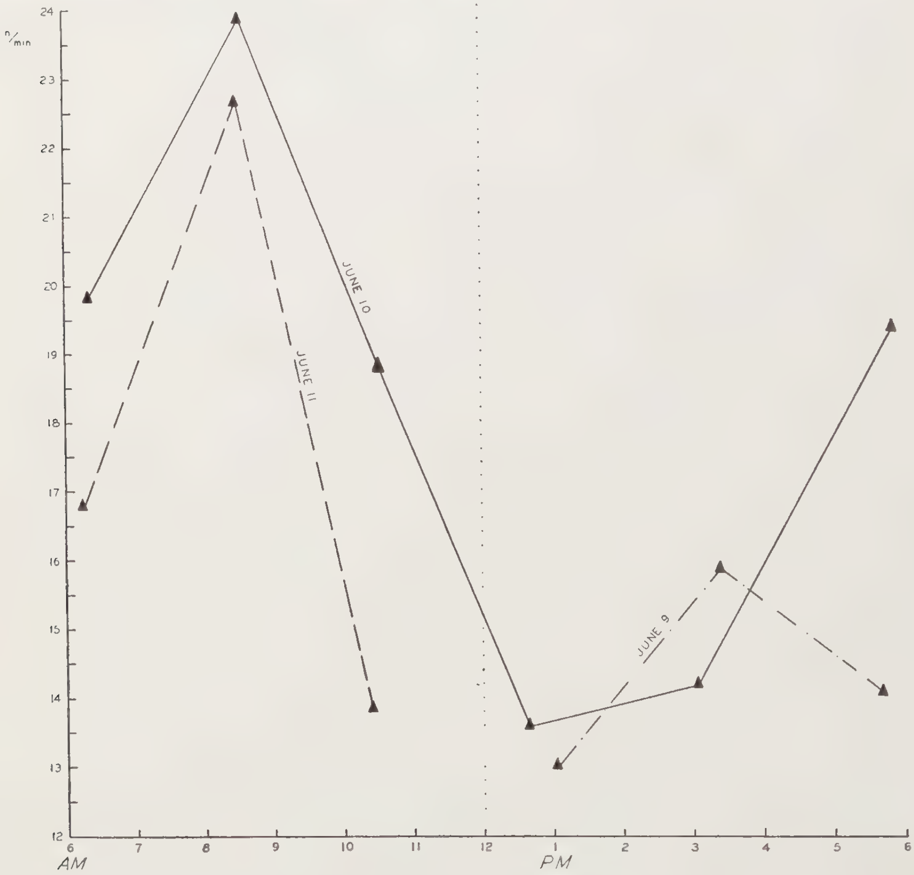


FIG. 4. The average number of female *Colias* counted per minute at various times of the day in the Imperial Valley alfalfa field.

The relative numbers of the female *Colias* which were counted over the

San Joaquin Valley alfalfa field during the different times of the day are shown in Figure 3. The number of females counted per minute is plotted against time. It may be observed that the frequency is low early in the morning (it is zero before sunrise, since the butterflies do not fly at night or without sunlight), reaches a peak from 9:30 to 10:30 A.M., and then drops slightly. The maximum number per minute was obtained at 10:30 A.M. The drop thereafter continues until about 2:00 P.M., after which time the numbers remain fairly constant. A correlation will be observed between the 2:00 P.M. hour in this connection and the minimum drop in white frequency.

A similar graph for the Imperial Valley population illustrates the abundance of active females in that valley at different times of the day (Fig. 4). Here the numbers increase from sunrise until about 8:30 A.M., at which time they are at their maximum (twenty-three or twenty-four per minute). On June 11 these had reached a near minimum of fourteen per minute by 10:30 A.M., but not quite that early on June 10. Except in one case all afternoon numbers are close to the minimum. On June 10 the numbers went up just before sunset, and this was accompanied by a rise of the white female frequency.

Again there is a correlation between the time when the population activity reaches its minimum for the day and the time when the white female frequency reaches its minimum. This time is 10:30 A.M. or soon after.

One may now observe the similarities and differences between the San Joaquin Valley curve and the Imperial Valley curve. Both have their maxima in the morning. The maximum of the Imperial Valley comes before that in the San Joaquin Valley (8:30 A.M. in contrast to 10:30 A.M.), and the minimum comes much earlier (about 10:30 A.M. in contrast to about 2:30 P.M.). These facts are relevant to the following discussion.

COMPARATIVE VARIATION IN THE ACTIVITY OF THE DIMORPHIC FEMALES

Two kinds of fluctuations in these populations have now been described: first, the variation in the relative frequencies of the two color phases; and second, the variation in the actual abundance of the females themselves at different times of the day. These are interconnected in the following way. The relative frequencies of the two color phases will not change if the rise and drop in activity of each is proportional. A rise in the frequency of the white phase can occur either by increasing the numbers of the active white-phase females as compared with those of the orange phase, or, conversely, by decreasing the number of the orange-phase females as compared with those of the white phase. A comparison of the numbers per minute of each phase at each period will show which of these is responsible for the observed result at any one time. Data for these are presented in Figures 5, 6, and 7 for the San Joaquin Valley, and 8 and 9 for the Imperial Valley (Table IV).

In Figures 5, 6, and 7 the numbers of females (white or orange) per minute are plotted against time. They show the relative abundance of the

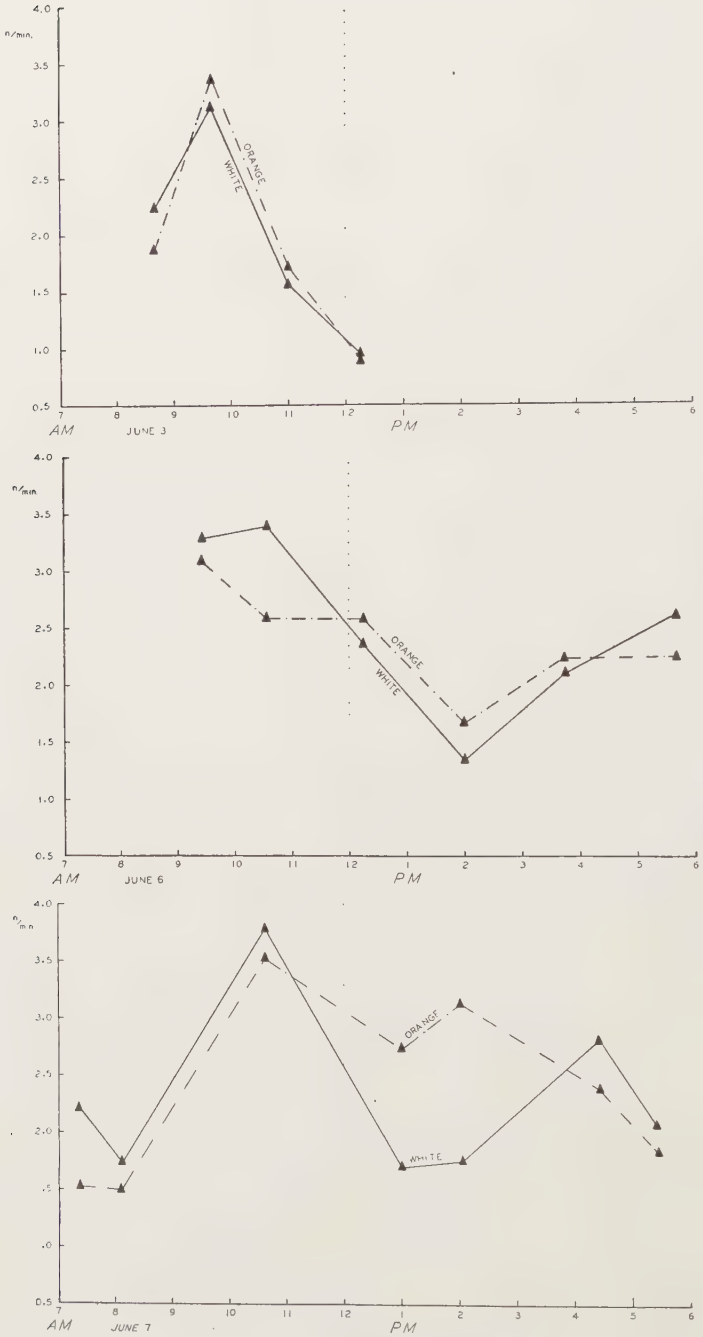


FIG. 5, 6, 7. A comparison of the relative proportions of orange and white phase females counted per minute at various times of the day at the San Joaquin Valley alfalfa field. Figure 5 represents June 3, Figure 6 represents June 6, and Figure 7 represents June 7.

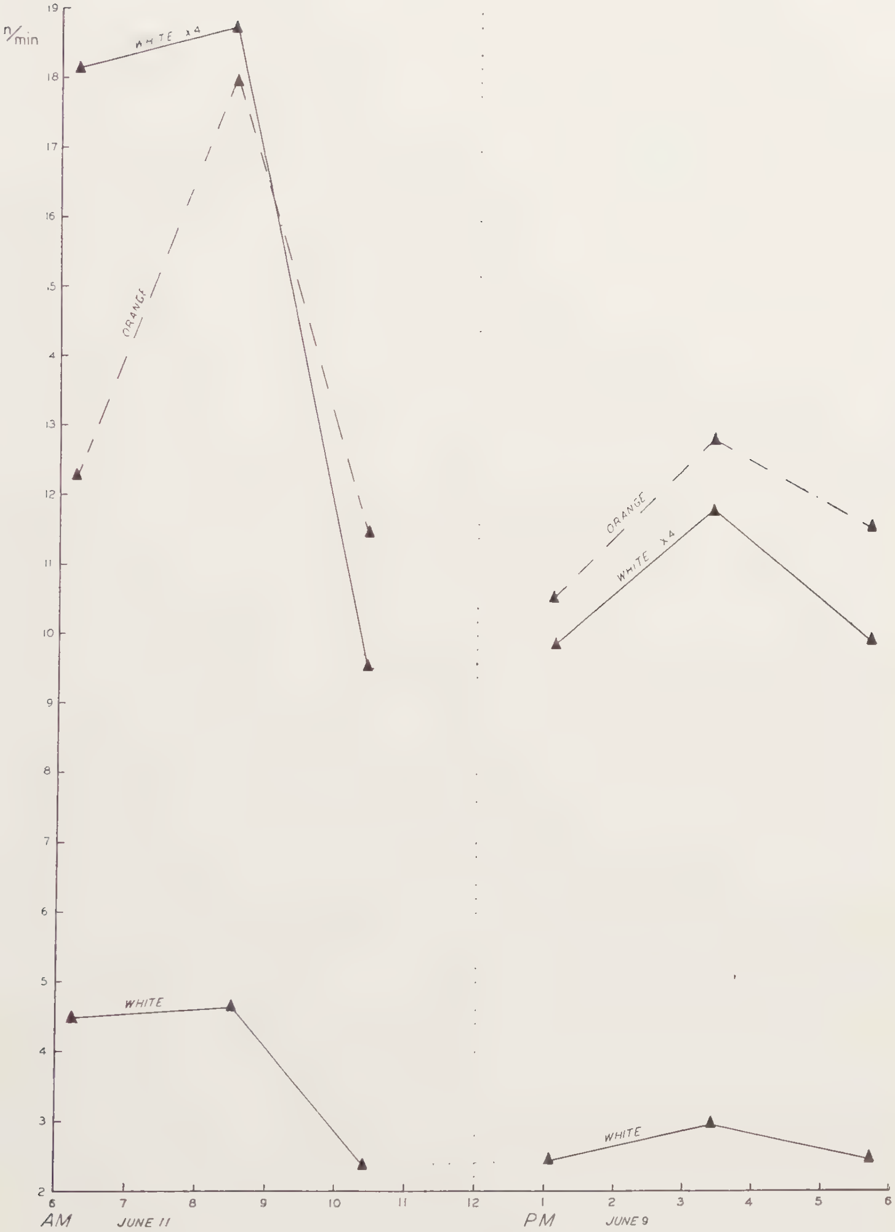


FIG. 8. A comparison of the relative proportions of orange and white phase females counted per minute at various times of the day in the Imperial Valley alfalfa field. The numbers of white females have been given in actual numbers (below) and with an increase proportionally to the white (above). This chart gives the data for June 9 and 11, 1946.

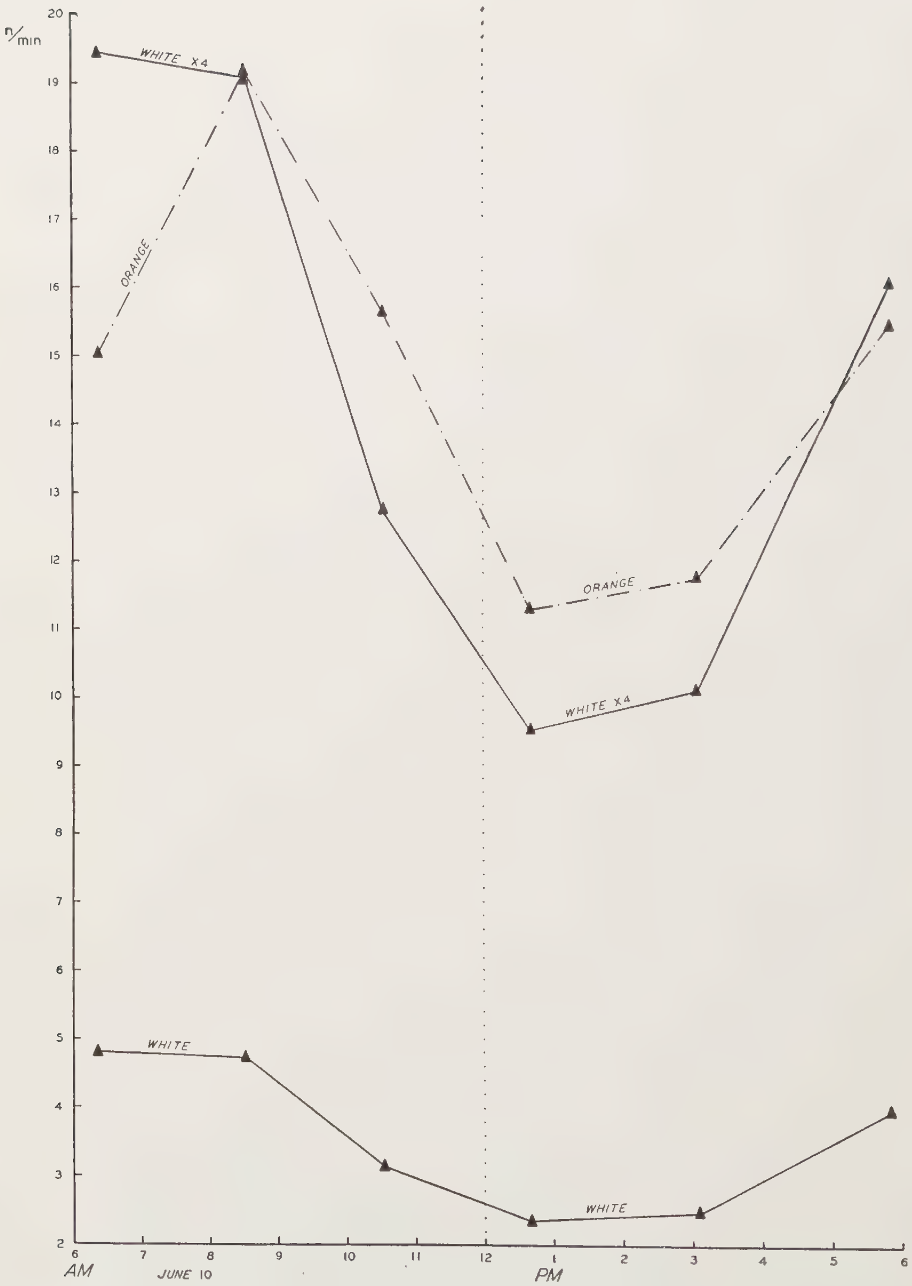


FIG. 9. The information for Figure 8 applies here also, but the data are for June 10, 1946.

white as compared with the orange females at any given time. Each indicates the same trend, namely, greater prevalence of white females as compared with orange in the early morning. For June 6 and 7 an increase in both the white and orange females is indicated up to 10 or 10:30 A.M., but the orange females seem to increase at a more rapid rate than do the white. This results in a lower white frequency. After 10:00 or 10:30 A.M. both orange and white phases decrease, but the white phase decreases more rapidly than does the orange. It has become proportionally less active than the orange phase. When the population has reached a midday minimum about 2:00 P.M. the white phase is considerably less active than is the orange, although both are less active than they were earlier. Thus, a drop in the white female frequency as shown by Figures 1 and 2 is not indicative of the mechanism behind the drop. Part of the drop occurs while both color phases are increasing in activity, and part occurs while both are decreasing in activity.

The rise of the white female frequency after 2:00 P.M. as shown in Figures 1 and 2 is caused by an increased activity of the white females with or without a corresponding increase in activity of the orange females.

The phenomenon is even clearer in the case of the Imperial Valley populations. The numbers of females counted per minute are again plotted against time; they are shown for June 9 and 11 on Figure 8 and for June 10 on Figure 9. Here, the proportional increase of one phase over another is difficult to compare because the actual frequencies are very different (18 per cent white, 82 per cent orange). Therefore, the numbers of white females in the population have been increased proportionally to an amount equal to that of the orange by multiplying the number of white phase females by a factor of 4.043. The curve is then plotted as before and can be compared directly with the actual numbers of the orange phase females (on the figures this curve is labeled white × 4; see also Table V).

As in the case of the San Joaquin Valley population, the white-phase females are relatively more abundant early in the morning than are the orange-phase females. The orange phase, however, increases more rapidly than does the white until about 8:30 A.M., at which time they both begin to decrease in abundance; this decrease continues until 10:30 A.M. or later. Again, the decrease of the white phase is proportionally more rapid than is that of the orange, and the result is a lowered white female frequency in the population. The straight line in Figure 2 showing the decrease of white female frequency is thus the result of differing activity of the two phases and includes both their periods of increase in activity and of decrease in activity.

The increase in white frequency before sunset on June 10 was caused by a general increase in activity, accompanied by a proportionally greater white-female increase. It is now desirable to determine, as well as possible, the factors of the environment which are involved in the differential activity of these color phases.

THE FACTORS OF THE ENVIRONMENT WHICH APPEAR CORRELATED WITH THE *Colias* ACTIVITY

The daily change in the frequencies in the color phases of *Colias* appears to be regulated by the physical factors of the environment. The more important factors are changes in temperature, in relative humidity, and in the intensity of the radiation of the sun at different times of the day. The differences in frequencies as observed could be the result of altered activity of the color phases with different combinations of high or low temperatures, relative humidities, or various intensities of the sun's radiation.

Solar Radiation

The radiation of the sun increases from dawn until noon and then decreases until sunset (Fig. 10), unless it is altered by cloudiness, by high humidity, or by dust. None of the latter factors were noticeably effective on the days the samples were made, except as mentioned below. I have no actual data for these particular localities.

The curves for the relative frequencies of the white and the orange color phases do not match the rise and drop of the relative solar radiation except early in the morning. The drop of white frequency indicated during the morning coincides with the increase in solar radiation, but the afternoon curve does not.

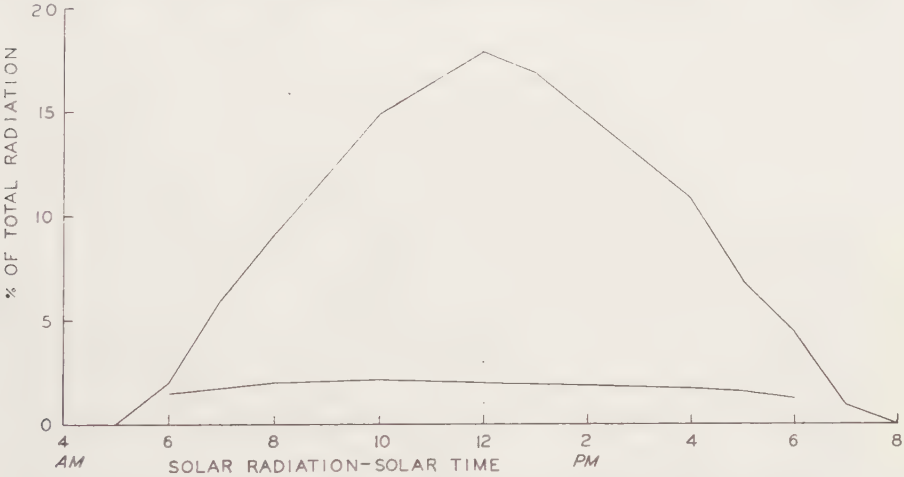
A comparison of the curves expected for solar radiation alone and the curves for the relative frequencies of white and orange females shows that these two curves are not congruous. The curve for solar radiation would reach the modal point at noon. The curves for the color phases show that a high point for orange is reached at sometime before or after 12:00 noon, depending on the locality. In the San Joaquin Valley (Fig. 1) the high point for orange (or, low point for white) is about 1:00 or 2:00 P.M. In the Imperial Valley (Fig. 2) the period of low frequency of white is more extended and is from about 10:00 A.M. until 3:00 or 6:00 P.M.

In the coastal area, Los Angeles, the high point for orange or low point for white frequency is sometime between 10:30 A.M. and 12:00 noon (Table I). For the Imperial Valley the frequency curves match the expected solar radiation curves in an increased orange frequency with increased solar radiation up to a maximum increase of 83 per cent orange (17 per cent white). Further increase in intensity of solar radiation is not followed by an increased orange frequency. With a drop in the intensity of the radiation in the afternoon there is no contemporaneous drop in the orange frequency indicated. Instead, the frequency remains at the maximum, at least until the radiation has become nearly negligible.

For the San Joaquin Valley the curve for the solar radiation matches that of the rise of the orange-phase frequency until 12:00 noon, at which time the orange frequency continues to rise despite the fact that the solar radiation decreased after noon. The decrease indicated in the orange-phase frequency began at around 2:00 P.M., but it does not indicate a drop as low as the minimum for the morning (Fig. 1).

The data for the coastal area are not detailed, but indicate an approximation to those of the San Joaquin Valley with the exception that the orange frequency drops to a low in the afternoon as great as that in the morning, and also that the high white frequency occurs before noon rather than in the afternoon (Table I).

It may be concluded, therefore, that variations in the solar radiation are, in a broad sense, contemporaneous with the variations in the color-phase frequencies, but that some other factors are responsible for wide fluctuations.



. FIG. 10. Curve of the percentage of total radiation received at the surface of the earth under normal conditions during a clear summer day (actual locality, Washington, D. C., somewhat north of the latitude of the populations being considered). The lower curve represents sky-radiation or indirect radiation (data from Kimball, 1924, through Brackett, 1936).

These butterflies do not fly at all at night, or when the direct sunlight is obscured by clouds. To that extent their activity is directly related to solar radiation.

Temperature

Changes in daily temperatures are closely related to changes in solar radiation, although the actual temperature at any given time depends upon many factors. Some of these are the nature and source of the local air mass, the length of the solar day, and the intensity of the wind. Generally speaking, the daily temperature rises as the solar day progresses. Even in the afternoon the temperature continues to rise until the heat from the decreasing solar radiation is surpassed by the loss of heat by the radiation of the earth, and then the temperature begins to drop. The sheltered interior San Joaquin and Imperial valleys illustrate these events especially well. Data for the two areas are presented in Figures 11 and 12. With a minimum temperature at about dawn (5:00 A.M.), there is a gradual rise throughout the day until the maximum is reached just before sunset (5:00 P.M.). The Los Angeles coastal area, which is exposed to the sea breezes, may be cooled early in the afternoon by oceanic winds, and the rising temperature may be retarded at about noon (Fig. 13).

The fit of the temperature curves to the color phase curves is again only a partial one. A rise of temperature in the San Joaquin Valley is contemporaneous with a rise in orange frequency until about 2:00 P.M., when the frequency of the orange phase drops despite continued rising temperature until 5:00 P.M. In the Imperial Valley the rise in temperature is contemporaneous with a rise in orange frequency until about 2:00 P.M., when the frequency of orange drops despite a rising temperature until 5:00 P.M. In the Imperial Valley the rise in temperature is contemporaneous with an increased frequency of the orange phase until about 10:00 A.M. At this time the frequencies of the white and orange phases reach an equilibrium at a minimum of white, even though the temperature continues to rise until 5:00 P.M. After 5:00 P.M. the data indicate that the frequency of the orange phase may or may not drop.

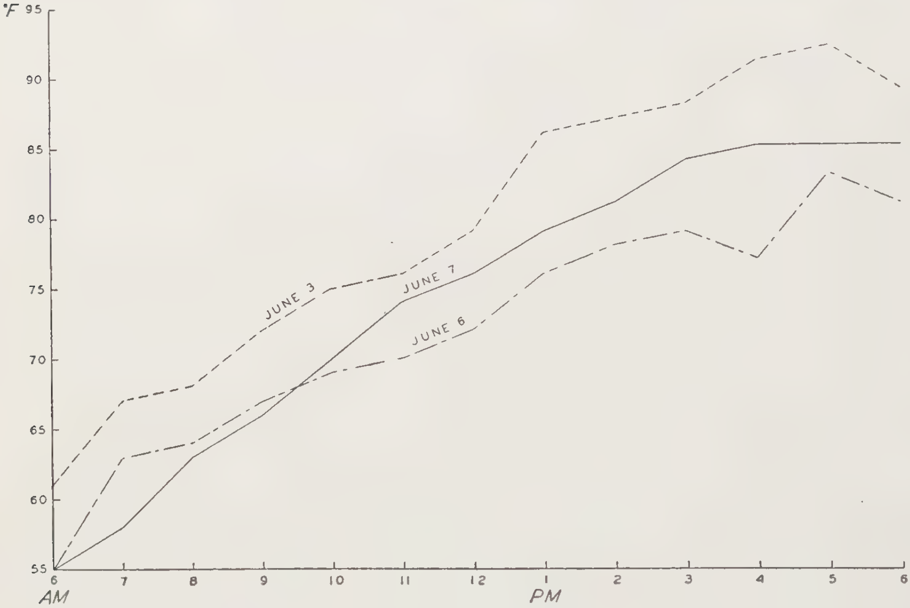


FIG. 11. Daily changes in temperature at a locality near Bakersfield, San Joaquin Valley, California, on the three days in which the analyses were made. Courtesy U. S. Weather Bureau.

At Rio Hondo (Los Angeles coastal area) the actual temperature fluctuations are complex owing to an air mass shift during the afternoon of the second day of the study. At that time there was a movement of hot, dry air from the interior toward the coast, thereby disrupting the normal daily rise and drop of temperatures. This air mass remained over the area until the following afternoon. For the three days September 20, 21, and 22, the following events occurred (Table I and Fig. 13): The lowest temperatures were recorded on the morning of September 20 (56° F. at 6:00 A.M.) with gradually rising temperatures until 5:00 P.M. (77° F.). On the same day the lowest frequencies of the white phase were recorded: 36.4 per cent, early A.M.; 25.0 per cent, late A.M.; and 35.5 per cent, P.M. Thus, there is no direct fit with the temperature data except in the morning. The mean temperature for the day (66° F.) is the lowest for the three days (69° F. and 72° F. for the next two days, respectively) and is contemporaneous with the lowest white-phase frequency for the three days (33.7 ± 3.5 per cent white as compared with 25.0 ± 3.8 per cent and 22.1 ± 4.3 per cent for the next two days). Temperatures on the second day (September 21) were higher than

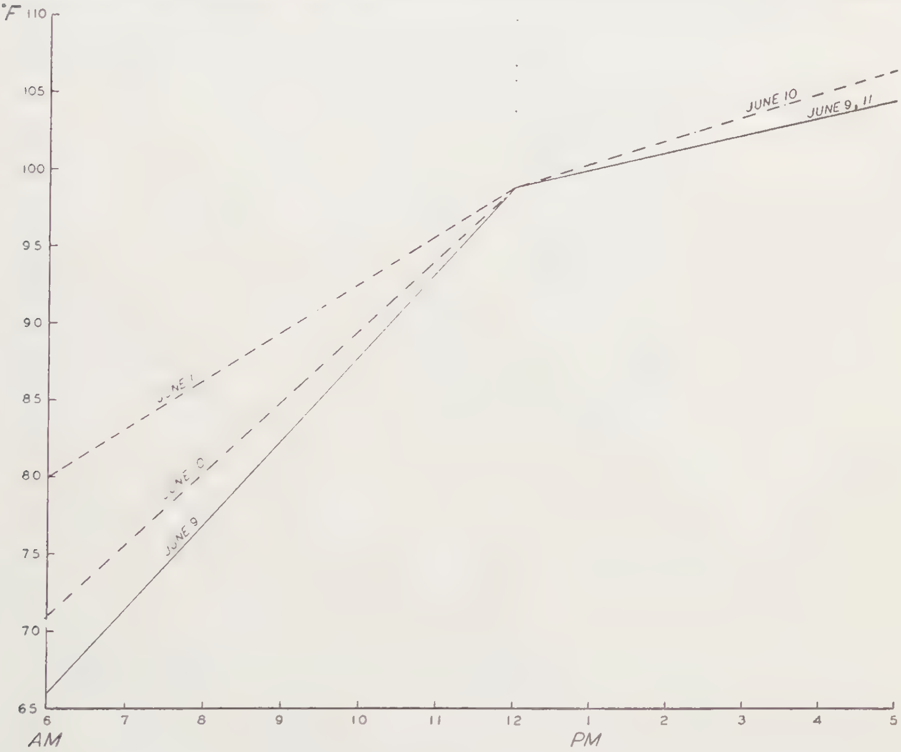


FIG. 12. Daily changes in temperature in the Imperial Valley near Brawley, California, on three days in which the analyses were made. (Noon temperatures were at Imperial Valley; high and lows were given at Brawley, but unspecified as to time; the times shown are indicated by other data of a similar nature from near-by weather stations.) Courtesy U. S. Weather Bureau.

on the first day, 56° F. at 5:00 A.M. and rising to 82° F. by 3:00 P.M. Except for the early morning the orange frequency fits that of the equivalent time of the preceding day. The aberrant morning frequency might be expected to be due to sampling errors or to the unusually warm late-afternoon temperatures of the preceding day. Again, there is no contemporaneous change of color phase frequencies and temperatures indicated. On the third day (September 22) the temperatures until 1:00 P.M. were higher than on the preceding days, 64° F. at 6:00 A.M. and rising to 81° F. at 1:00 P.M., but they dropped thereafter upon a shift in the air mass movement from seaward to normal landward again. The highest orange-phase frequencies are recorded for this day (82.4 per cent orange). Unfortunately, the frequencies were not tested that afternoon nor again until September 27.

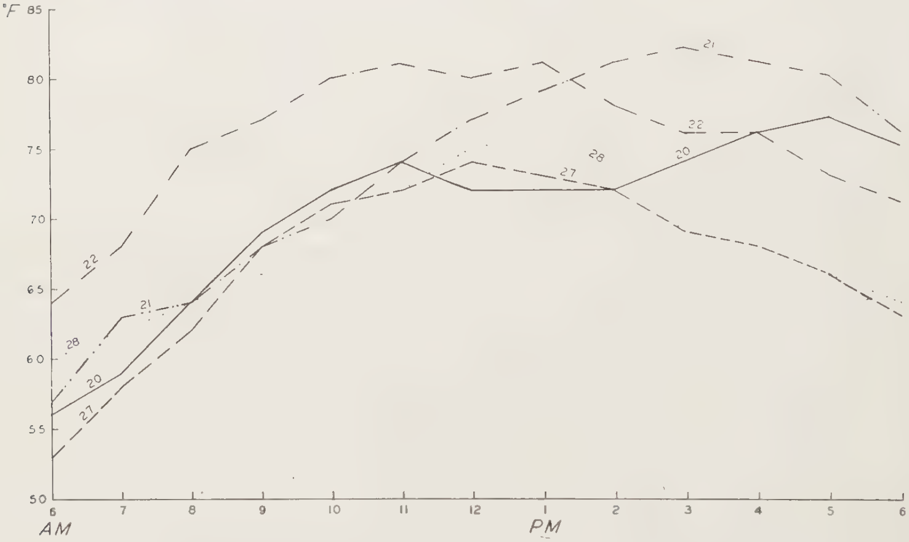


FIG. 13. Daily changes in temperature at Los Angeles, California, on the three days in which analyses were made. September 27 and 28 are shown for comparison. Courtesy U. S. Weather Bureau.

By combining the frequencies for the three days for the respective three periods of the day (Table I), it may be observed that the changes in color phase frequencies fit the temperature changes for the morning only and not for the afternoon. The rises in orange frequency and temperature are contemporaneous until noon, but not entirely so in the afternoon.

Consider again the relationship between the color phase frequencies and the temperature of each of the three localities. The orange phase frequency always rises until a certain time during the day in each of the three localities. This time is different in each locality, being about 10:00 A.M. in the Imperial Valley, about 2:00 P.M. in the San Joaquin Valley, and probably about 12:00 noon on the coast. Then the orange frequency rises no more and may even decrease, although the actual temperature continues to rise until sunset. It may be concluded, therefore, that changes in color phase frequencies are contemporaneous with the temperature changes during the morning but not during the entire day.

Humidity

In each of the three localities the relative humidity is generally inversely proportional to the temperature (Fig. 16), that is, the relative humidity drops as the temperature rises, to give a daily curve somewhat comparable, but reversed, to that of the temperature (Figs. 14 and 15). For this reason it would be impossible to differentiate between the effects of the temperature changes and those of the relative humidity.

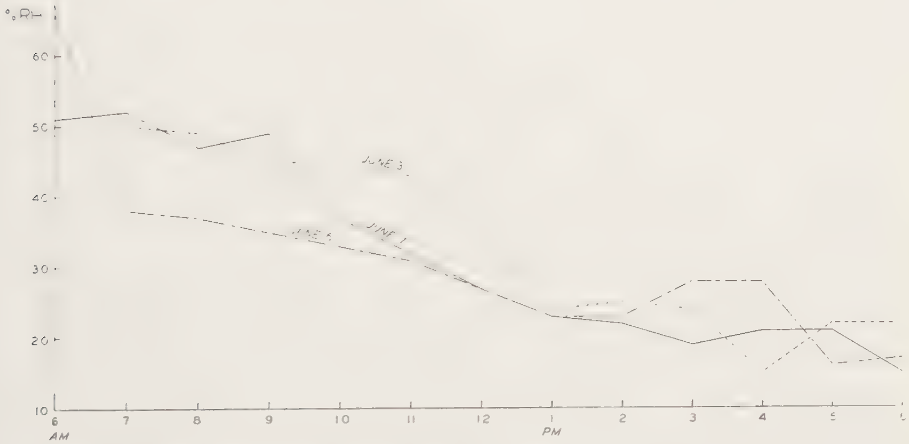


FIG. 14. Daily changes in relative humidity near Bakersfield, San Joaquin Valley, during the three days June 3, 6, and 7, 1946. Courtesy U. S. Weather Bureau.

On September 20, in the afternoon, the air mass at Rio Hondo (as was noticed earlier) changed from a humid coastal one to a dry interior one. The temperatures rose somewhat, but not enough to compensate for the decreased relative humidity. The following day, while the dry air was present and the temperatures rose very high, the white phase frequency dropped in the population.

At the time of the replacement of the coastal air mass by the continental one, it was observed that nearly all species of butterflies began a migration to the south and west (the direction of air mass movement). This migration was participated in by *Colias eurytheme,* and the butterflies became scarcer in the locality. It was this scarcity that accounted for the cessation of collecting on the afternoon of September 22. It is uncertain whether the drop in the white phase frequency on the morning of September 21 and 22 was related to this movement. Similar changes in air mass are correlated with butterfly migration (Williams and Bishara, 1929; Hovanitz, 1944*b*), but their effects on changing gene frequencies (or phenotype activity) are unrecorded. Daily decreases of the white frequency in these populations, recorded before (Hovanitz, 1944*b*), will be considered more fully in another paper.

It may be concluded that the data do not allow one to differentiate between the direct effects of rising temperatures and the inverse effects of decreasing relative humidities on the comparative frequencies of the color phases of *Colias*. There is reason to believe that the decreasing humidity is responsible for a different activity of both phases and may be responsible for a relative difference in their activity.

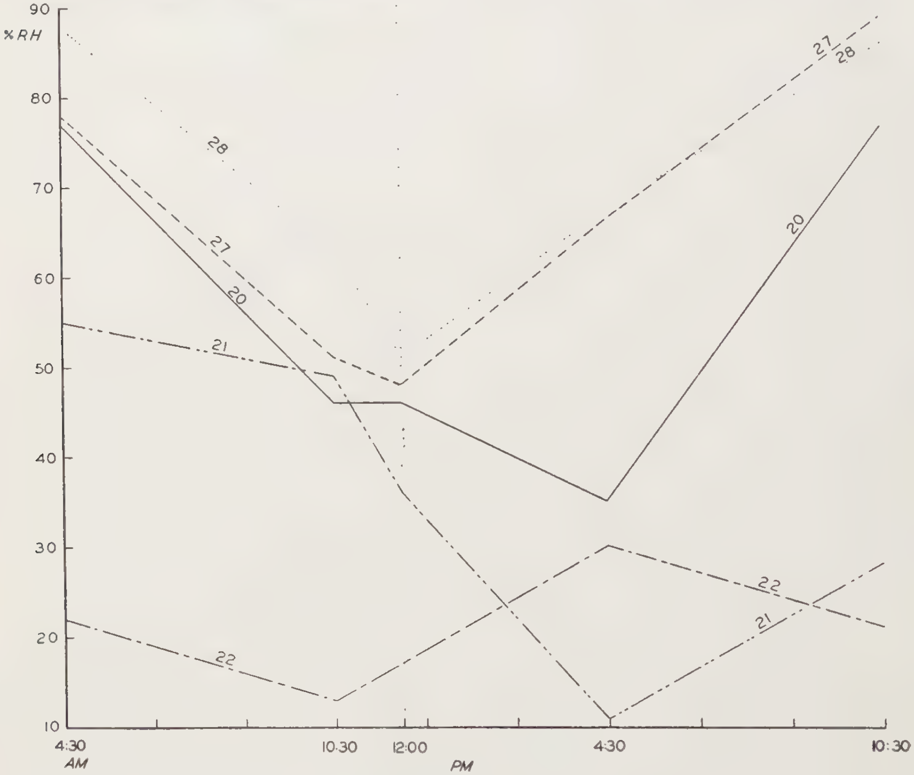


FIG. 15. Daily changes in relative humidity at Los Angeles, California, during the five days September 20, 21, 22, 27, and 28, 1941. Courtesy U. S. Weather Bureau.

CORRELATION BETWEEN SOLAR RADIATION, TEMPERATURE, AND ACTIVITY OF THE FEMALES

Were a sufficiently large quantity of data available, it should be possible to determine the combination of meteorological conditions which are optimum for butterfly activity. Multiple correlations might be determined for any combination of independent meteorological variables with activity of the adults as the dependent variable. From these, conversely, it should be possible to infer the activity of similar butterflies as a function of any similar combination of meteorological variables.

The information available is small, however, and extensive statistical analysis does not seem justified. Nevertheless, the following tests were made.

TABLE VI

Data Used for Computations and Charts on Multiple Correlations

	Total n/min.	Total Adjusted to Imperial Valley	Temp. ° F.	Solar Radiation Per Cent	R.H. Per Cent	w	z
			x	y			
San Joaquin Valley							
June 3							
8: 40 A.M.	4.09	15.27	72	55	46	.891	−16.36
9: 40 A.M.	6.49	24.23	75	70	45	1.414	2.75
11: 00 A.M.	3.27	12.21	76	79	43	.712	13.12
12: 15 P.M.	1.87	6.98	80	83	32	.407	22.60
June 6							
9: 25 A.M.	6.38	23.82	68	70	34	1.390	−6.84
10: 35 A.M.	5.97	22.29	70	75	32	1.301	.90
12: 15 P.M.	4.93	18.40	75	83	26	1.074	15.75
2: 00 P.M.	3.02	11.27	78	75	23	.658	11.86
3: 45 P.M.	4.36	16.28	77	55	28	.950	−9.51
5: 40 P.M.	4.86	18.14	81	20	16	1.059	−39.03
June 7							
7: 25 A.M.	3.74	13.96	60	37	50	.815	−50.80
8: 05 A.M.	3.23	12.06	63	45	47	.704	−38.69
10: 35 A.M.	7.29	27.21	73	75	34	1.588	5.01
1: 00 P.M.	4.44	16.57	76	83	23	.967	17.12
2: 05 P.M.	4.88	18.22	81	75	22	1.063	15.97
4: 25 P.M.	5.20	19.41	85	37	21	1.133	−16.55
5: 25 P.M.	3.95	14.75	85	20	18	.861	−33.55
Imperial Valley							
June 9							
1: 05 P.M.	12.98	....	100	80	9	.758	47.00
3: 25 P.M.	15.82	....	103	56	8	.924	27.11
5: 45 P.M.	14.06	....	105	20	8	.821	−6.15
June 10							
6: 25 A.M.	19.80	....	73	20	30	1.157	−49.99
8: 35 A.M.	23.82	....	83	55	24	1.391	−1.29
10: 35 A.M.	18.74	....	93	79	18	1.095	36.41
12: 40 P.M.	13.58	....	100	83	13	.793	50.00
3: 05 P.M.	14.18	....	104	60	12	.828	32.48
5: 25 P.M.	19.28	....	107	20	9	1.126	−3.41
June 11							
6: 15 A.M.	16.75	....	81	20	35	.978	39.03
8: 30 A.M.	22.64	....	85	55	29	1.322	1.45
10: 25 A.M.	13.80	....	94	79	24	.806	37.78

The total numbers of butterflies counted at successive periods of the day are given in Table VI for both the Imperial and the San Joaquin valleys. Included in this table also are meteorological data for the same periods of the day which have been interpolated from Figures 10–12.

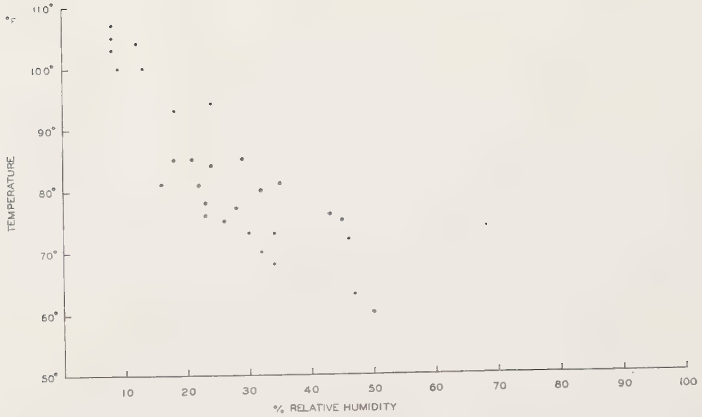


FIG. 16. Correlation between relative humidity and temperature in the combined data of the Imperial and San Joaquin valleys.

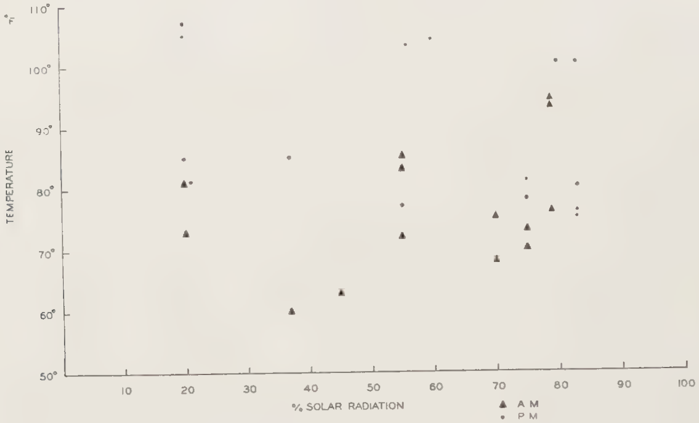


FIG. 17. Correlation between temperature and solar radiation in the combined data of the Imperial and San Joaquin valleys. Differences between the morning and the afternoon.

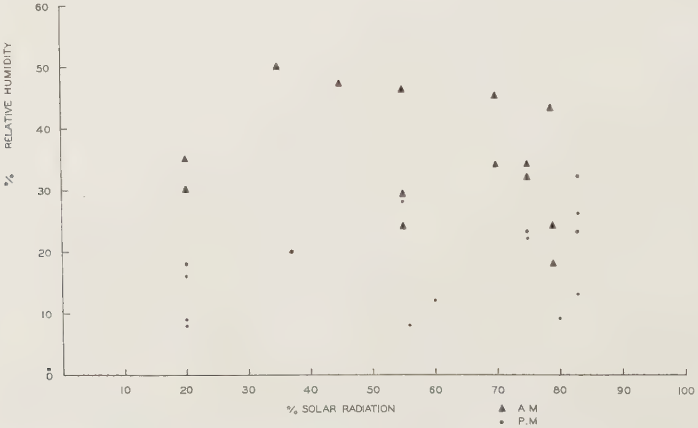


FIG. 18. Correlation between solar radiation and relative humidity in the combined data of the Imperial and San Joaquin valleys. Differences between the morning and the afternoon.

Correlations between the following factors might be made: solar radiation, temperature, relative humidity, and time of day. Only those which are relatively independent of the others, however, may give information which is new. Temperature and relative humidity in the Imperial and San Joaquin valleys are very closely related (Fig. 16). Therefore, the relative humidity data were not used in the correlations. Figures 17 and 18 indicate that the other factors, such as temperature and solar radiation at a particular time of day, may also be interrelated if the data were sufficiently detailed. These details, however, generally were considered too fine to be used with the rough data collected on the butterflies themselves.

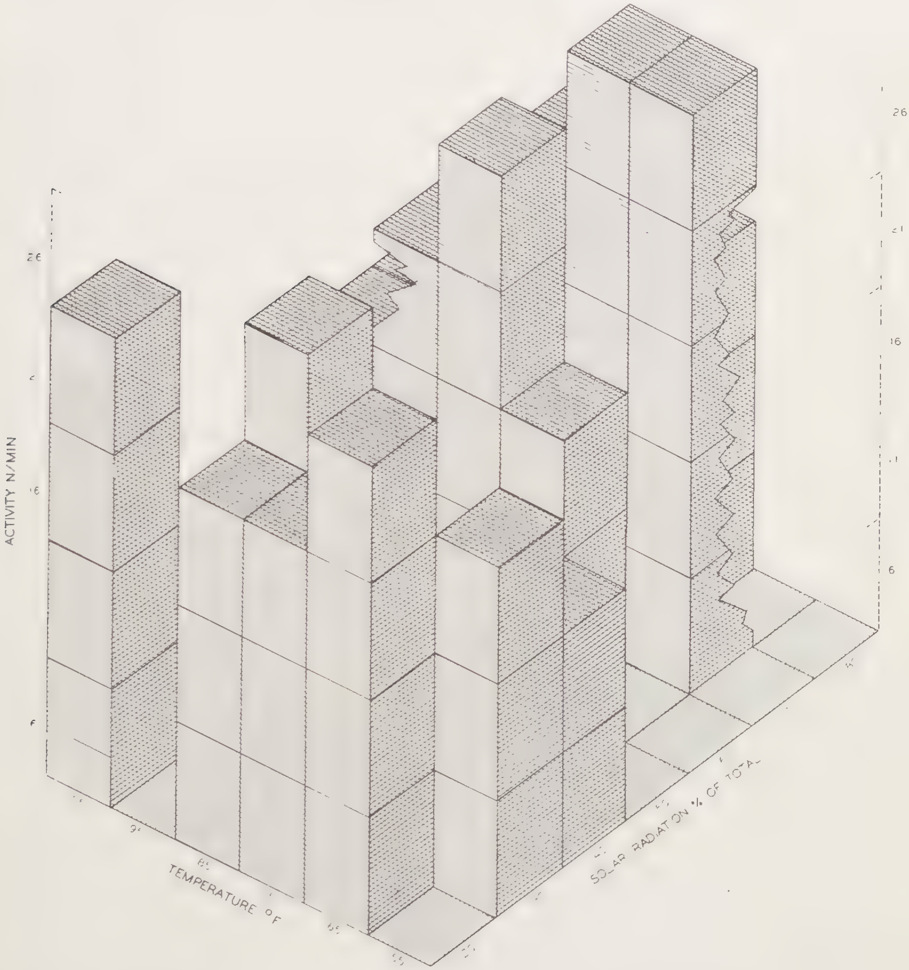


FIG. 19. The relation between solar radiation, temperature, and activity of female *Colias* in the Imperial and San Joaquin valleys, California.

A three-dimensional model of the correlation between solar radiation, temperature, and the activity of the females is given in Figure 19. The elevation of each block indicates the number of females counted per minute for a given combination of the two factors, solar radiation and degree of temperature. Not all points could be indicated on the figure because of hidden blocks or duplicating blocks. It can be seen, however, that the combinations ranging from high solar radiation with low temperature to low solar radiation and high temperature are more closely correlated with greater activity than are the combinations of low temperature with low solar radiation and high temperature with high solar radiation.

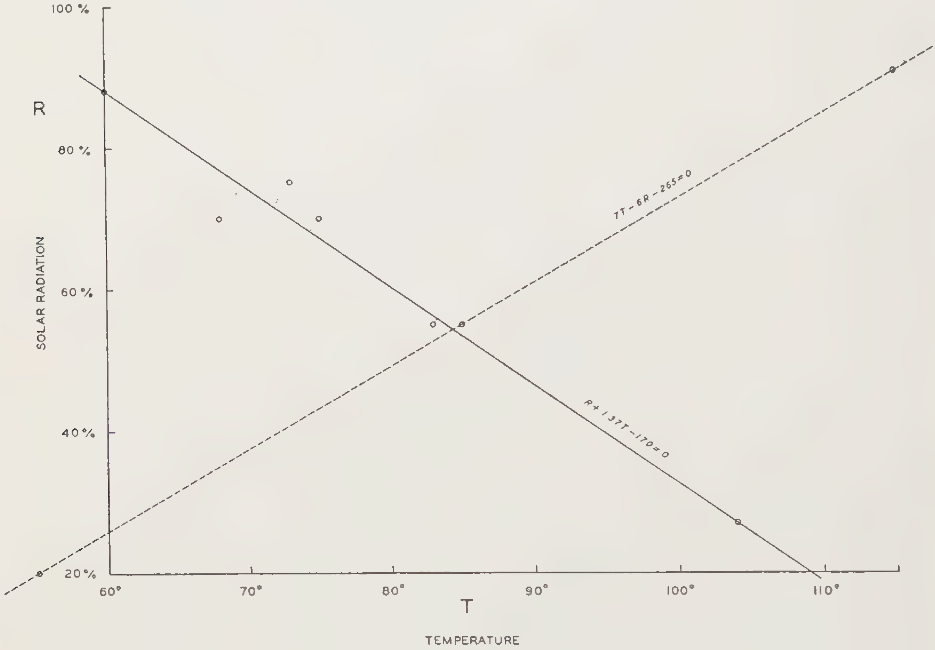


FIG. 20. Line found by selecting five peak activities for butterflies and plotting these points against solar radiation (*R*) and temperature (*T*) fitted to make the sum of the squares of the distances from the points to the line a minimum (solid line). Also, line connecting the hypothetical points 55°, 20 per cent, and 115°, 90 per cent, and thus separating the actual points into two classes according as they are above or below this line (dashed).

C. C. Craig, of the Statistical Research Laboratory of the University of Michigan, has tested the significance of this correlation in two ways. First, five peak activities were selected, one for each of five days. These peak activities occurred at the temperature–solar radiation combinations 75°, 70 per cent; 68°, 70 per cent; 73°, 75 per cent; 83°, 55 per cent; and 85°, 55 per cent. A peak activity line was fitted to the points determined by these pairs and plotted on Figure 20. Since both temperatures and solar radiation are subject to error, the line was fitted to make the sum of the squares of perpendicular distances from the points to the line a minimum. The line so found has the equation $R + 1.37T - 170 = 0$.

Then setting,

$$z = R + 1.37T - 170,$$

z so found is proportional to the distance of any point (T, R) from the line. The actual distance on Figure 20 is

$$\frac{z}{\sqrt{1 + 1.37^2}} = \frac{z}{1.70},$$

which is positive for points above the line and negative for points below it. The values of z are shown in Table VI.

A test was then made of the hypothesis that there is a tendency for activity to be proportional to the distance of the point (T, R) from the line.

TABLE VII

VALUES OF w CLASSIFIED AS ABOVE OR BELOW THE LINE CALCULATED BY THE FORMULA $7T - 6R - 265 = 0$ AS SHOWN IN FIGURE 20

$-w$	$+w$
.891	1.059
1.414	1.133
.712	.861
.407	.924
1.390	.821
1.301	1.157
1.074	.828
.658	1.126
.950	.978
.815	
.704	$N = 9$
1.588	
.967	$m = .9874$
1.063	
.758	Student-Fisher $t = .00748$ with 26 degrees of freedom
1.391	
1.095	
.793	
.806	
$N = 19$	
$\overline{m} = .9883$	

This is done by finding the coefficient of correlation between activity and $|z|$, the absolute value of z. First, the recorded activities were all reduced to percentages of the average activity for the locality in which they were observed. These values were designated by w and are given in Table VI. Then the correlation sought is $r_{w|z|}$, which proves to have the value 0.499. This value is significant at the level of .01, and thus the hypothesis tested is not refuted by the data.

The line of best fit for predicting w's from $|z|$'s has the equation $w + 0.00798|z| = 1.178$. Geometrically interpreted, this is the equation of two planes equally inclined to the T, R plane which intersect in the peak activity line, and it has been shown that there is a real tendency for the activity points with co-ordinates (T, R, w) to cluster about these planes.

The hypothesis was then tested that there may be a lower activity of the butterflies in the combinations of high temperature and low solar radiation than there is in the combinations of low temperature and high solar radiation. This test was made by dividing the points (T, R) into classes according to whether they were above or below (in the T, R plane) the line joining the points (55°, 20 per cent, and 115°, 90 per cent) shown dashed in Figure 20. The equation for this line is $7T - 6R - 265 = 0$. When the co-ordinates of any point (T, R) are substituted in $7T - 6R - 265$ the result is $\gtrless 0$ according to whether the point is below or above this line. For example, taking the first pair of values (72°, 55 per cent), one has $7 \times 72 - 6 \times 55 - 265 < 0$, and the point is above the line, as one may verify by plotting it. In Table VII the values of w are divided into two classes, those for points above the line and those for points below the line. One point, namely 85°, 55 per cent, proved to be on this line. The means of the two groups are 0.988 and 0.987, respectively, which is a rather minute difference as shown by the calculation of the Student-Fisher t-test (Table VII).

TABLE VIII

MULTIPLE CORRELATIONS BETWEEN TOTAL NUMBER OF BUTTERFLIES COUNTED AT A GIVEN TIME AND SOLAR RADIATION AND TEMPERATURE, BASED UPON THE ASSUMPTION THAT ALL POINTS LIE IN A SINGLE PLANE

Locality and Date	Multiple Correlation Coefficient	Significance
Imperial Valley		
June 9	.619	$P > .05$
June 10	.864	$P > .01$
June 11	.503	$P > .05$
San Joaquin Valley		
June 3	.105	$P > .05$
June 6	.228	$P > .05$
June 7	.283	$P > .05$

An assumption that there may be a simple linear relationship between activity as a dependent variable and solar radiation and temperature as independent variables was tested first. Numbers of butterflies per minute were correlated with solar radiation and temperature, but for each of the six days separately. These data are indicated in Table VIII. Only the Imperial Valley, June 10 data gives a high correlation which seems significant. This is .864 with $P > .01$.

The interpretations which can be drawn from these data are that a correlation between the activity of the butterflies and a combination of meteorological factors of the environment seems indicated and that solar radiation and temperature may be the two major factors involved. Activity of the butterflies is greatest with the combinations of the factors high solar radiation and low temperature, and low solar radiation and high temperature. Calculated on the basis of two inclined planes intersecting at the peak points, the other points do not deviate significantly from these two planes if drawn so that the sum of the squares of the distances of the points away from these two planes is at a minimum. The data do not fit nearly as well to a single plane so constructed to all the points.

DISCUSSION

The preceding data illustrate the differential activity during the day of two female color phases of *Colias eurytheme* when the two color phases occur together in the same wild population. The two color phases are the "normal" orange female form, somewhat similar to the males, and the dimorphic white female form. The white form is genetically differentiated from the orange form by a single dominant gene (Gerould, 1923; Hovanitz, 1944*c*). The two forms represent freely interbreeding parts of the same population. They are not sexually isolated units which happen to occur in the same place, as is true for the yellow and orange forms (Hovanitz, 1943*b*).

The frequencies of these two forms are different in geographically separated populations. The white form is most abundant in northern populations and less abundant in southern populations (Hovanitz, 1944*a*). Fluctuations have been recorded also in the relative frequencies of these phases in single populations. These may be either seasonal changes, as at Mono Lake, California, or variations during a single brood emergence, as in the San Fernando Valley or San Joaquin Valley, California (Hovanitz, 1944*a*).

The data presented in this paper illustrate fluctuations in the frequencies of these color phases in a population during the course of a single day. These fluctuations have been interpreted as variations in the relative activity of the two phases under the influence of the daily weather variations during the day.

Similar daily fluctuations were found at three localities, the San Joaquin Valley, the coastal area near Los Angeles, and the Imperial Valley. All localities are in California and are on a north to south axis as stated. The average frequency of the white females in these three places is approximately 50 per cent, 30 per cent, and 15 per cent, respectively.

In each of these localities the white frequency was highest early in the day and was lowest in each toward the middle of the day. The time when the minimum was reached was different in each population, but in general it was earlier in the day in the southern population and later in the day in the northern population. In some instances the frequency of white increased again in the afternoon, but was not usually as high as it was in the morning.

The mechanism of the changing white frequency is a differential activity of the two forms at different times of the day. Early in the morning the white females were proportionally much more active than were the orange females. Later in the day the orange females increased in activity, but the white females did not. This lowered the frequency of white. Later still both forms decreased in activity, but the white females decreased much more than did the orange. This continued the observed decrease in the frequency of the white females. In the afternoon an increase was indicated in both forms on some of the days.

The activity of the females was greatest at some point during the morning in all populations. It was earlier in the day in the more southern populations, namely, at 8 : 30 A.M. in the Imperial Valley and at 10 : 30 A.M. in the San Joaquin Valley.

A period of minimum diurnal activity occurred about the middle of the day. This period was also earlier in the southern population than in the northern, namely, onset at 10 : 30 A.M. in the Imperial Valley and 2 : 00 P.M. in the San Joaquin Valley.

Rough correlations were tested between the changes in the meteorological factors of the environment and the changing color-phase frequencies. Correlations were tested with such factors as solar radiation, temperature, humidity, and time of day. No one of these factors could be shown to be wholly responsible for the changes which occurred. It seems highly probable, however, that a certain combination of these conditions is responsible, but the data are apparently not sufficiently detailed to allow one to determine the combination with accuracy.

Correlations calculated between certain meteorological factors of the environment and the total activity of the females seem to indicate that the activity of the adults may be related to specific combinations of intensity of solar radiation and degree of temperature. With more precise, or a greater quantity of, data significant differences might be found between the requirements of the two color phases.

SIGNIFICANCE

The differences in the amount of activity of butterflies of the two color phases during the day indicate that the adults as well as the earlier stages of development of the dimorphic color phases in *Colias* have physiological differences in behavior. These conclusions were indicated by the seasonal and brood changes in frequency as reported in other papers. That these physiological behavior differences are controlled by the same pair of allelomorphs that control the white and orange colors seems likely because the alleles controlling both phases are segregating freely in the same population, and the recombinations of each generation would tend to disorganize any linkages which might arise between different genes.

The data are significant in two ways: (1) they indicate that the mechanism by which allelomorphs can be selected by environmental factors may be indirect, and (2) they show that some physiological differences in behavior are directly correlated with color differences.

The first point is an important postulate connected with the mechanisms of evolution. Apart from an almost impossible random establishment of a new gene in a large population, the only mechanism available which would be very effective in increasing the frequency of an allele in a population is selection of that allele by the environmental selection of the genotypes which carry it. Populations which are interbreeding cannot long remain genetically differentiated without an excess of selection pressure acting against the normal tendency toward panmixia. The field data show that the frequencies of the color phases are different in adjacent populations, and that the individuals of these populations do interbreed. One may conclude, therefore, that selection must be acting differentially on these populations in order to alter the proportions of the phases in each.

Populations in the north (Hovanitz, 1944*a*) and at high elevations (Hovanitz, 1945) have a higher white frequency than do populations in the south or at low elevations. One climatic characteristic is common to both elevated and northern areas. This characteristic is low temperature. If the selective agent acts upon the color phases during the adult stage, it might be expected that the white individuals would be relatively more abundant during the colder part of the day than during the warmer (provided that white reflects significantly more radiation than does orange). It is clear that the results shown in this paper support this conclusion. The white phase individuals are more abundant during the cooler part of the day than they are during the warmer part. Wherever this is true, it would follow that geographical localities having a cooler climate would be more advantageous to the white form than to the orange, and the white form would supplant the orange by contributing more to each succeeding generation.

The second point is also important for an understanding of the results of evolution. Some parallel color variations in butterflies, other insects, plants, and animals in various regions are logically explained by differences in behavior which are correlated with the color (Hovanitz, 1941). If these color differences were caused by the same physiological action in development as the behavior differences, then an environmental factor selecting for a behavior quality will also select for a color variety. This concept has been illustrated as of possible occurrence in diverse groups of organisms (Hovanitz, 1947).

The present data indicate that environmental selection for a physiological behavior quality in *Colias* could be responsible for a selection of color varieties. The adults themselves differ in amount of activity as a response to various combinations of meteorological factors acting upon them.

SUMMARY

1. Comparative differences are indicated in the amount of activity of two *Colias eurytheme* female color phases at different times of the day. These color phases, the orange and the white, are genetically controlled by a single pair of alleles.

2. The frequency of the white-female phase in the total number of females which were flying in a field early in the morning is higher than it is later in the day. The frequency is less toward the middle of the day and may or may not rise again in the afternoon.

3. The changing frequencies during the day have been shown to be due to a differential activity of the two phases. The white phase is relatively more abundant very early in the day. Thereafter, the orange phase increases in abundance, but with a proportionally lower white increase so that the frequency of white decreases. Then, both phases decrease, but the white continues to decrease proportionately greater. Both again may increase in the afternoon.

4. The period during the day for the most and for the least diurnal activity is indicated to be earlier in a more southern locality than in a more northern one.

5. Partial correlations occur between these frequency changes and the meteorological factors, such as temperature, solar radiation, and humidity, at different times of the day.

6. The total activity of the butterflies has been tested as dependent upon various temperature and solar radiation combinations. Activity is greatest along a peak line ranging from high temperature and low solar radiation to low temperature and high solar radiation. The existing data do not deviate significantly from two planes formed by intersection at this peak activity line and sloping toward the low temperature and low solar radiation and high temperature and high solar radiation directions.

7. There is no significant difference in activity when the points as indicated in the preceding paragraph are divided into two classes, low temperature and high solar radiation and high temperature and low solar radiation.

8. The points formed as above by the various combinations of temperature and solar radiation as independent variables correlated with activity as a dependent variable show a very poor linear correlation.

9. The significance of these data to the mechanisms of evolution are discussed. Two points seem especially significant: (1) a color gene might be selected by an environmental factor owing to a physiologically different activity which the organism manifests under certain environmental conditions, (2) physiological differences in behavior are directly correlated with color differences.

LITERATURE CITED

Brackett, F. S.
1936 Measurement and application of visible and near-visible radiation. *In* Biological Effects of Radiation, ed. by B. M. Duggar. 1: 123–209.

Gerould, J. H.
1923 Inheritance of white wing color, a sex-limited (sex-controlled) variation in yellow pierid butterflies. Genetics, 8: 495–551.

HOVANITZ, W.

1941 Parallel ecogenotypical color variation in butterflies. Ecology, 22: 259–84.

1943*a* The nomenclature of the *Colias chrysotheme* complex in North America. (Lepidoptera: Pieridae.) Amer. Mus. Novit., 1240: 1–4.

1943*b* Hybridization and seasonal segregation in two races of a butterfly occurring together in two localities. Biol. Bull., 85: 44–51.

1944*a* The ecological significance of the color phases of *Colias chrysotheme* in North America. Ecology, 25: 45–60.

1944*b* The distribution of gene frequencies in wild populations of *Colias*. Genetics, 29: 31–60.

1944*c* Genetic data on the two races of *Colias chrysotheme* in North America and on a white form occurring in each. *Ibid.*, 29: 1–30.

1945 The distribution of *Colias* in the equatorial Andes. Caldasia (Bogotá, Colombia), 13: 283–300.

1947 Occurrence of parallel series of associated physiological and morphological characters in diverse groups of mosquitoes and other insects. Contrib. Lab. Vert. Biol., Univ. Mich., 32: 1–24.

MS Population size and dispersal in *Colias*.

KIMBALL, H. H.

1924 Records of total solar radiation intensity and their relation to daylight intensity. Monthly Weather Rev., 52 (1): 473–79.

WILLIAMS, C. B., and I. BISHARA

1929 The seasonal abundance of four common butterflies in Egypt. Bull. Royal Ent. Egypt, 13: 85–92.

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